The Role of Oxidative Stress in Noise-Induced Hearing Loss

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Abstract: **Modern research has provided new insights into the biological mechanisms of noise-induced hearing loss, and with these new insights comes hope for possible prevention or treatment. Underlying the classic set of cochlear pathologies that occur as a result of noise exposure are increased levels of reactive oxygen species (ROS) that play a significant role in noise-induced hair cell death. Both necrotic and apoptotic cell death have been identified in the cochlea. Included in the current review is a brief review of ROS, along with a description of sources of cochlear ROS generation and how ROS can damage cochlear tissue. The pathways of necrotic and apoptotic cell death are also reviewed. Interventions are discussed that target the prevention of noise-induced hair cell death: the use of antioxidants to scavenge and eliminate the damaging ROS, pharmacological interventions to limit the damage resulting from ROS, and new techniques aimed at interrupting the apoptotic biochemical cascade that results in the death of irreplaceable hair cells.**

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Introduction

Noise-induced hearing loss (NIHL) is a significant source of hearing loss for people in industrial societies. Currently, prevention of NIHL focuses on developing hearing conservation programs, which include modifications of the noise source, mandating the use of hearing protection devices, frequent hearing screening of populations at risk, education on the causes of NIHL, and ways to prevent it (Dobie, 1995; Lusk, 1997). Although such actions remain the most effective measures currently available to the audiological community in the fight against NIHL, there are still a number of people working in construction or the military where conventional hearing conservation programs are difficult to operate. In these environments, alternative or complementary approaches are needed. Recently, research on the cellular bases of NIHL has led to new avenues for protection through the use of prophylactic drugs. The development of these drugs was made possible because of new insights into the biological mechanisms of NIHL, which in turn provided rational cues for developing the drug therapies.

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Effects of Noise on the Cochlea

We have known for years that noise causes a broad set of physical changes in the major cellular systems in the cochlea, changes that lead to temporary threshold shift (TTS) and permanent threshold shift (PTS). Figure 1A is a schematic of the organ of Corti with the major points of vulnerability highlighted in red. Normal stereocilia are seen in Figure 1B. Figure 1 (C and D) shows that stereocilia can be broken, fused, or have broken tip links that lead to loss of structural integrity (Liberman, 1987; Pickles, Osborne, & Comis, 1987; Tsuprun, Schachern, Cureoglu, et al., 2003).

Additionally, the ability of the stereocilia to act as mechanoelectrical transducers is reduced due to a loss of permeability of protein transduction channels in the cell membrane surrounding the stereocilia (Patuzzi, 2002). The tips of the stereocilia on outer hair cells (OHC) can also be removed from their points of insertion with the tectorial membrane, leading to a loss of sensitivity (Nordmann, Bohne, & Harding, 2000). There is a window of time between the disconnections of the tips of the largest stereocilia from the tectorial membrane in which the tips can reattach (Nordmann et al., 2000). TTS may partially be the consequence of the stereocilia damage and repair (Patuzzi, 2002; Saunders & Flock, 1986).

Because the cochlea is a mechanical analyzer that codes frequency into a tonotopic organization, its mechanical structure and impedance are critical for maintaining faithful representation of sound vibrations along the cochlear partition. Damage to pillar cells (Fig. 1, E and F) has also been observed after impulse noise (Salvi, Hamernik, & Henderson, 1979) and high-level continuous noise (>115 dB SPL). The loss of the pillar cells interferes with the local impedance of vibration. Consequently, mechanically coded vibration of the organ of Corti may be disrupted by loss of the pillars. In addition, the loss of the pillars may also contribute to the loss of OHC.

High levels of noise can also lead to swelling and rupturing of the dendritic terminals of the auditory nerve afferent fibers (Spoendlin, 1971) through the

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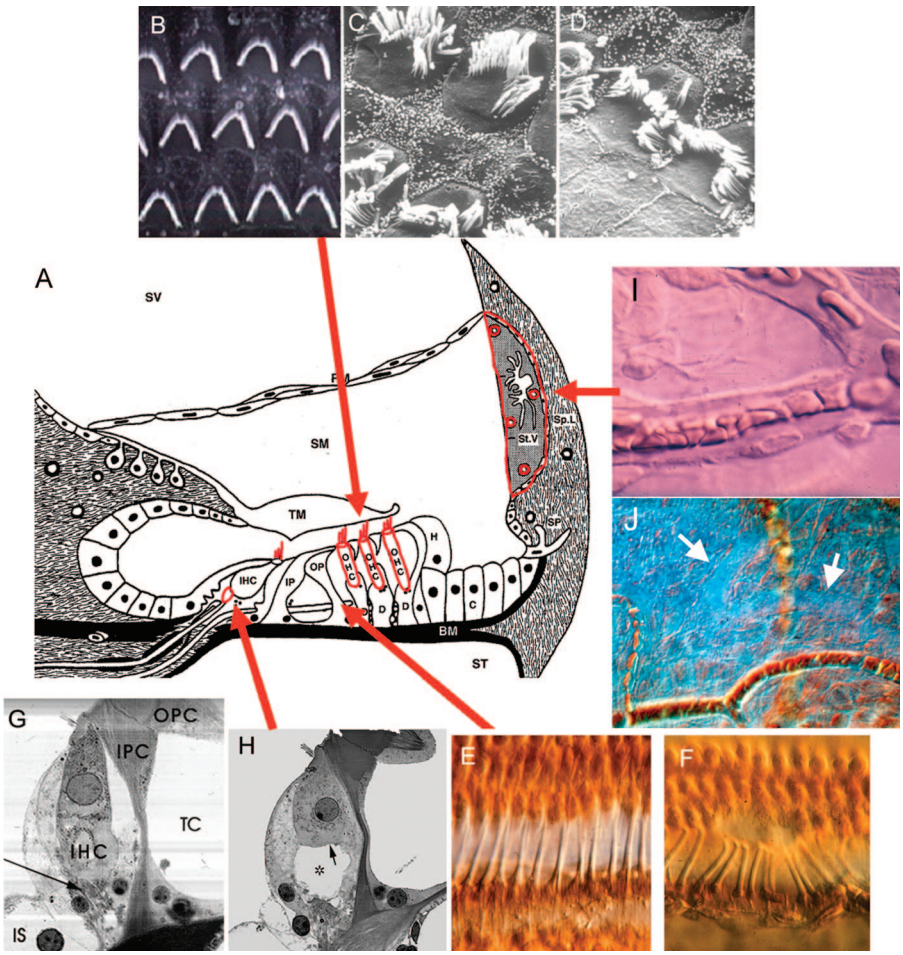


Fig. 1. ***A,*** **Normal cross section of a turn of the cochlea. Points of vulnerability to high-level noise are highlighted in red.** ***B,*** **Scanning electron microscopic picture of a normal outer hair cell stereocilia.** ***C*** **and** ***D,*** **Scanning electron microscopic pictures of outer hair cell stereocilia after exposure to noise.** ***E,*** **Normal outer pillar cells.** ***F,*** **Outer pillar cells 15 minutes after exposure to impulse noise.** ***G,*** **Normal inner hair cell–auditory nerve fiber junctions.** ***H,*** **An extreme form of kainic acid–induced pathology similar to pathology induced by noise.** ***I,*** **Normal capillary in lateral wall of the cochlea.** ***J,*** **An avascular channel. All of the images are from chinchilla cochlea samples. The chinchilla has been used extensively in noise-induced hearing loss research programs and other auditory research programs because there is much similarity between the audiograms of humans and of chinchillas.**

mechanism of glutamate excitotoxicity (Puel, Ruel, Gervais, d'Aldin, et al., 1998; Pujol, Puel, Gervais, d'Aldin, et al., 1993). Glutamate is the excitatory neurotransmitter that acts at the synapses of the inner hair cells (IHC) with the VIIIth nerve fibers (Fig. 1G). During high-level noise exposure, the IHC are highly active, leading to the release of large amounts of glutamate into the synapses with the type I fibers of the VIIIth nerve. The levels of glutamate in the synapses can overstimulate the glutamate receptors on the postsynaptic cells. The result is the condition of excitotoxicity, characterized by swelling of the postsynaptic cell bodies and dendrites (Kandel, Schwartz, & Jessel, 2000). The swelling is a result of postsynaptic ion influx into the VIIIth nerve terminals that occurs due to excessive excitation from the glutamate (Pujol, Puel, d'Aldin, et al., 1990). Application of a glutamate blocker limits the dendritic damage and reduces noise-induced threshold shift, suggesting that the damage is contributing to noise-induced threshold shift (Puel, d'Aldin, Saffiedine, et al., 1996). Over time, the swollen or ruptured dendrite terminals appear to recover and begin to function normally, suggesting that this type of damage may also contribute to TTS (Robertson, 1983). The hypothesis that noise-damaged afferent auditory nerve synapses can recover is supported by studies using treatment with kainic acid, a glutamate analogue. Studies in our laboratory (Zheng, Henderson, Hu, et al., 1997) have shown that kainic acid placed on the round window crosses into the cochlea and causes swelling and eventual temporary loss of dendrites (Fig. 1H).

The cochlea is richly supplied with blood vessels at the spiral ganglion and along the lateral wall (stria vascularis and spiral ligament). High level noise can lead to acute swelling of the stria vascularis (Wang, Hirose, & Liberman, 2002), swelling that is associated with loss of intermediate cells of the stria. The swelling disappears over time, but the loss of intermediate cells is permanent (Hirose & Liberman, 2003). Therefore, the overall size of the stria vascularis shrinks as a long-term result of noise exposure. Associated with damage to the stria is a short-term reduction in the endocochlear potential (EP) (Ide & Moirmitsu, 1990). After very high-level noise exposures, the EP changes can be permanent but are largely restricted to areas of extreme hair cell (HC) and stereocilia damage. The changes in EP, even if permanent, do not appear to have a

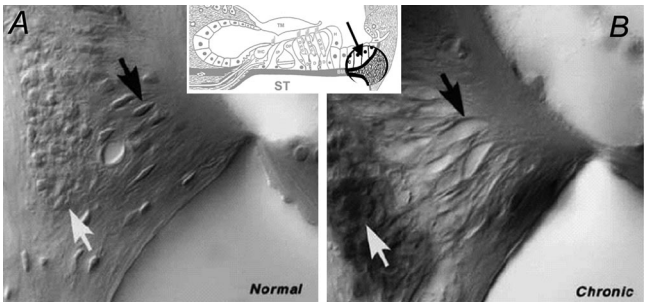


Fig. 2. Type IV fibrocytes from the outer sulcus of a normal mouse cochlea (*A*) and from a cochlea with chronic cochlear pathology (*B*) evaluated 2 wks after a 2-hr octave band noise (8 to 16 kHz) of 94-dB SPL. From Wang, Hirose, & Liberman (2002).

major impact on hearing loss from noise (Hirose & Liberman, 2003).

Partly associated with damage to the lateral wall blood vessels, high level noise exposure can reduce cochlear blood flow (CBF). The extent of the effects of noise on CBF appear to be heavily influenced by the length and intensity of the noise exposure (Hultcrantz, 1979; Lamm & Arnold, 2000; Perlman & Kimura, 1962; Prazma, Vance, Bolster, et al., 1987; Shaddock, Hamernik, & Axelsson, 1985; Yamane, Nakai, Takayama, et al., 1995). Any reductions in cochlear blood supply, even if transient, could induce threshold shifts and lead to damage to vital cochlear tissue. Figure 1I shows a normal capillary packed with red blood cells. After noise exposure, the capillaries of stria vascularis can be found empty. Over a period of several days, some of these damaged capillaries degenerate, leaving avascular channels (Fig. 1J). Decrease of CBF may be a contributing factor to noise-induced cochlear pathology, a topic that will be discussed in greater detail in a later section of this review.

Ion balance across the apical membrane of HC, critical for normal hearing, is also susceptible to disruption by noise. Recently, Spicer & Schulte (1996) have reported that critical levels of potassium ions (K^+) in endolymph are maintained by a complex pathway that includes cycling through the OHC (particularly during stimulation), then through fibrocytes in the outer sulcus region of the lateral wall, and finally, back to stria vascularis. Wang, Hirose, & Liberman (2002) reported a loss of type II and type IV fibrocytes in the region where the OHC were most heavily damaged by a noise exposure (loss of type IV fibrocytes marked with black arrows in Fig. 2, A and B). Loss of the type II and type IV fibrocytes could potentially disrupt the cycling of the K^+ ions through the cochlea. This disruption could lead to Na^+-K^+ pump in the stria vascularis that works to produce the endolymphatic potential. Yet, currently the impact of fibrocyte damage on hearing loss is not clear.

Noise can damage most of the cell populations in the cochlea, but the OHC are the most prominent pathological target. The OHC at the basal end of the cochlea are the most vulnerable and are lost first, even with a broad band noise exposure. From a functional perspective, loss of OHC leads to elevated hearing thresholds (up to 40 to 60-dB threshold when only the OHC are missing), along with loss of cochlear frequency tuning. The classic pattern of early noise-induced hearing loss in the audiogram includes a notch centered at 4 kHz of approximately 30 dB that is the consequence of a lesion of OHC centered at the corresponding region of the organ of Corti (Fig. 3A). However, with more severe noise exposures (Fig. 3B), the pathology spreads to include IHC death, loss of auditory nerve fibers, and damage to stria vascularis (Bohne, 1976).

Impulse noise exposures can damage the cochlea by causing direct mechanical damage, as well as pathology described above (Fig. 1). Depending on the intensity of the impulses, the organ of Corti can be ripped from the basilar membrane. Pillar and Hensen's cells can be destroyed, or their structural contributions in the organ of Corti can be compromised. In addition, cell junctions between HC, Deiters' cells, and Hensen's cells can be broken (Hamernik, Turrentine, & Roberto, 1986). Interestingly, the lesion of HC death immediately after impulse noise exposure can be fairly small, but the lesion grows over the period of 2 to 30 days after exposure (Hamernik et al., 1986). The cause of the prolonged expansion of the lesion has been attributed to a number of pathologies, including entry of endolymph through holes in the reticular lamina into the cortilymph that bathes OHC (Ahmad, Bohne, & Harding, 2003; Bohne & Rabbit, 1983).

Oxidative Stress and Hair Cell Death

The catalog of pathological changes (Fig. 1, Fig. 2, and Fig. 3) is the result of exposure to high-level noise, but a question still remains about what active mechanisms at the cellular level are triggering HC death. We are now learning that oxidative stress is a major part of the answer.

In the mid 1990s, a number of studies emerged that showed the appearance of increased reactive oxygen species (ROS) and toxic free radicals during and after noise exposure. Free radicals are molecules with an unpaired electron, making them capable of altering the electron arrangements in stable molecules. [See tutorial box 1 for more detail on ROS. Interested readers may consult *Free Radicals in Biology and Medicine* by Halliwell & Gutteridge (1999), a highly readable discussion of free radicals and ROS in a biological context.] ROS are oxygen-based molecules

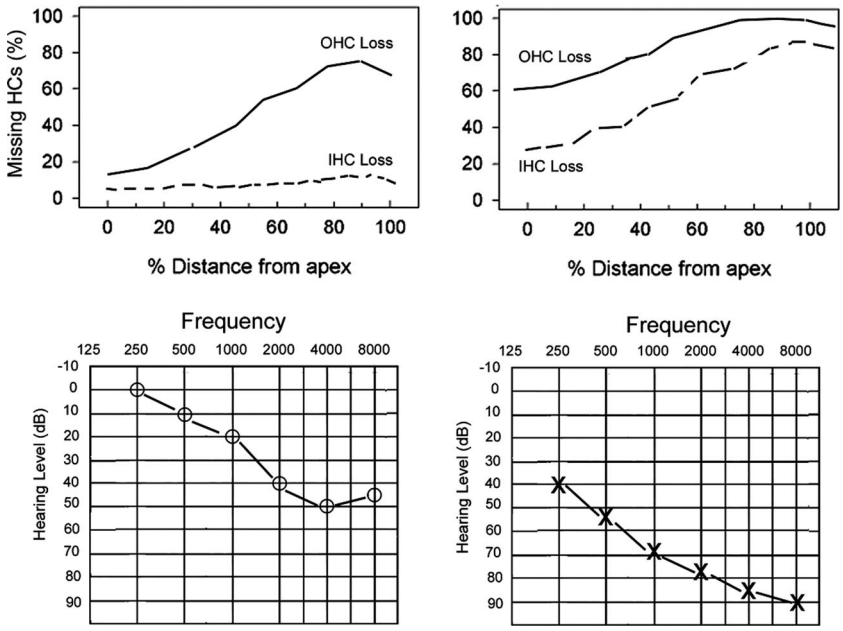


Fig. 3. Hair cell death (cochleograms) and their associated audiograms. Sample A has a 60 to 70% lesion of outer HC in the basal, or high-frequency, end of the cochlea, with a mild hearing loss of up to 50 dB in the affected region. Sample B has a more extreme lesion involving both outer and inner hair cells, associated with a much larger hearing loss affecting a broader frequency range.

that either act as free radicals themselves (superoxide ($O_2^{\cdot -}$), the hydroxyl radical ($OH^{\cdot}$), and the peroxynitrite radical ($ONOO^{\cdot -1}$)) or are readily capable of generating free radicals [hydrogen peroxide (H_2O_2), ozone (O_3)] (Halliwell & Gutteridge, 1999). In 1995, Yamane et al. observed increases in superoxide ($O_2^{\cdot -}$) along marginal cells of stria vascularis, along with empty strial capillaries, after an exposure to high-intensity (120 to 125 dB SPL) rock 'n' roll music. However, after 6 hrs, the superoxide was greatly reduced, and the capillaries were filled with red blood cells. Ohlemiller, Wright, & Dugan (1999) found increases in the hydroxyl radical ($OH^{\cdot}$) after exposure to 110-dB SPL broad band noise. Ohinata, Miller, Altschuler, et al. (2000a) found evidence of increased lipid peroxidation after a 115-dB SPL 4-kHz octave band noise. Lipid peroxidation consists of a series of reactions through which free radicals and ROS can break down lipid molecules, such as those in the membrane of a cell. It is a self-perpetuating reaction (Halliwell & Gutteridge, 1999) and may be one of the ways in which free radicals and ROS can cause continued cell damage after the noise exposure has ended.

The Yamane et al. (1995) and Ohlemiller et al. (1999) studies link ROS activity with damaging noise exposure, but their approaches did not allow a direct localization of the ROS to the OHC. Nicotera, Henderson, Zheng, et al. (1999) exposed chinchillas to a 4-kHz octave band of noise for 2 hrs. Immediately after the exposure, the subjects were anesthetized and perfused with dichlorofluorescein, a fluorescent dye that stains for a chemical product of lipid peroxidation (see tutorial box 2 for an explanation of functional staining of the cochlea). An example of the results is shown in Figure 4. The first panel of Figure 4 shows a slight background staining in a normal-hearing, nonexposed, cochlea. The second panel shows the noise-exposed cochlea 30 minutes after the noise. Notice the systematic labeling along the bottom of the three rows of the OHC (indicated with circles). The strong fluorescence showing the labeled ROS at the OHC corresponds to a site in the cochlea that is vulnerable to noise (Nicotera, Henderson, Zheng, et al., 1999).

How Do ROS Damage the Cochlea?

The presence of ROS within noise-damaged cochlear tissue raises the issue of whether the ROS lead to cochlear damage or if the ROS are a product of damaged/dying cells. Application of chemicals into the perilymph that are known to generate superoxide and the hydroxyl radical led to significant increases in the compound action potential (Clerici &

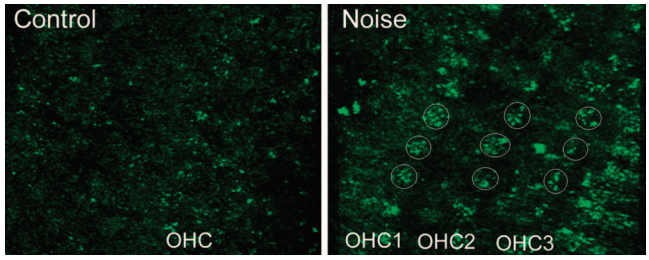


Fig. 4. Chinchilla organs of Corti labeled fluorescent green with dichlorofluorescein (DCF) after no treatment (control) or 15 minutes after impulse noise exposure (noise) of 50 pairs of 150-dB pSPL simulated M-16 gunfire. Note the regular labeling in the OHC region. See tutorial box 2 for more information on DCF.

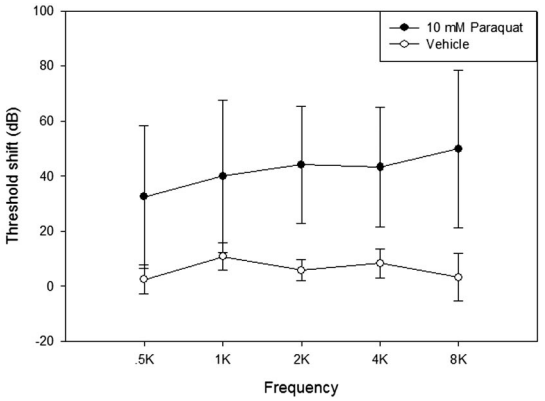


Fig. 5. Mean permanent threshold shifts (PTS) induced by application of a drop of 10 mM paraquat (dark circles) onto the round window membrane of chinchillas. Open circles are controls that received only vehicle solution. Error bars are ±1 SD.

Yang, 1996). Another direct assessment of the potential of superoxide to damage cells can be obtained by using paraquat, which creates superoxide from molecular oxygen (O_2) and the molecule NADPH (an electron carrier molecule that donates the electron to convert oxygen to superoxide). Paraquat delivered to the round window at a dose of 10 mM can induce significant PTS (Fig. 5) and HC loss (Bielefeld, Hu, Harris, et al., 2005). The fact that paraquat alone leads to HC death and hearing loss suggests that increased superoxide is a sufficient cause of hearing loss.

HOW ARE ROS FORMED IN THE COCHLEA AS A RESULT OF NOISE?

The exact origins of the increased ROS observed in the cochlea are currently somewhat speculative. The electron transport chain that occurs in the mitochondria as part of aerobic respiration is a major source of superoxide. The electron transport chain is a series a of reactions in which electrons are moved from one carrier to another to release energy to be used in the synthesis of ATP (adenosine triphosphate), the major energy source molecules for cells. Each movement of the electrons releases energy that is used to convert the molecule ADP (adenosine diphosphate) to ATP through a process called phosphorylation. As the electrons move from carrier to carrier, superoxide is formed as an intermediate molecule. When the mitochondria are using more and more oxygen to meet increased cellular demands for energy, more and more superoxide is generated as an unwanted byproduct, due to the inefficiency with which the mitochondria must work (Halliwell & Gutteridge, 1999) (see tutorial box 3 for a brief overview of the activity within the mitochondria). The OHC, in part because of their motility, are known to be highly demanding of energy (Thalmann, Miyoshi, Kusakari, et al., 1975), and high-level noise exposure places especially high demands on the mitochondria to generate large amounts of energy through aerobic respiration. During a noise exposure, the electron transport chain of the mitochondria uses large amounts of oxygen, which can then create large amounts of superoxide generated as an unwanted byproduct. The increased superoxide can then react with other molecules to generate higher levels of other ROS in the cochlea (Halliwell & Gutteridge, 1999) (see tutorial box 1).

Yamane et al. (1995) hypothesized that the initial deposit of superoxide that they observed along the marginal cells of the stria vascularis after noise was the result of overdriving of the mitochondria in the absence of available oxygen and that the oxygen deficiency was due to a noise-induced reduction in CBF, or ischemia (see tutorial box 4 for more detail on ischemia as a generator of ROS). Ischemia decreases the cochlear oxygen supply, with the consequence that the phosphorylation process in the mitochondria (in which ATP is generated from ADP and oxygen) becomes more inefficient. This inefficiency leads to the generation of superoxide (as discussed above). After ischemia, blood flow can recover, and there is a restoration of the normal levels of available oxygen (known as reperfusion). The reperfusion of available oxygen can lead to even more superoxide formation. Many studies of noise-

Reactive oxygen species (ROS) are a broad class of oxygen-based molecules. Included in the category of ROS are oxygen-based free radicals. Free radicals are molecules with an unpaired electron in their outer shell. Free radicals are powerful in their ability to destabilize other molecules and break down molecular interactions, leading to cell and tissue damage. Targets for ROS damage include: proteins, lipids, and DNA. In addition to free radicals, the class of ROS molecules includes some molecules that are not free radicals themselves, but will readily generate free radicals. Hydrogen peroxide is an example of this type of ROS.

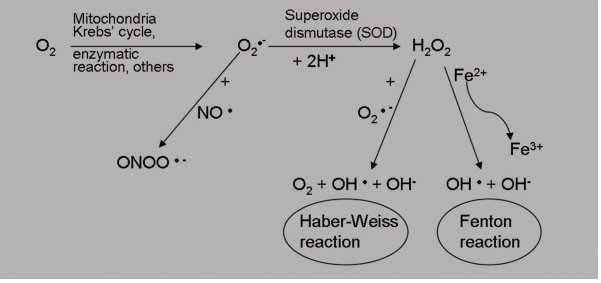
Superoxide ($O_2^{\bullet -}$): Molecular oxygen with an additional unpaired electron. By itself, superoxide is not a powerful ROS, but it is easily converted into more powerful ROS like the hydroxyl radical or peroxynitrite.

Hydrogen peroxide (H_2O_2): Another fairly docile ROS, but can be readily converted into the powerful hydroxyl radical.

Hydroxyl radical ($OH^{\bullet}$): A very dangerous ROS that can appear as the product of Fenton reactions between hydrogen peroxide and ferrous iron molecules (Fe_3^+). The ferrous iron cations are readily available inside of cells, and in some cases, when cells die, they can spill their intracellular contents into the extracellular space. When this spillage occurs, the ferrous iron cations become readily available for use in the Fenton reaction as a generator of $OH^{\bullet}$.

Peroxynitrite ($ONOO^{\bullet -}$): Another potent ROS that is the product of a reaction between superoxide and nitric oxide ($NO^{\cdot}$), a common molecule in the body.

ROS are generated through a number of processes and reactions in the body. Below is a diagram showing some of the important reactions and relationships between various ROS that create dangerous ROS.



Tutorial box 1.

Functional "Staining of Cochlea": The cochlear tissue and pathology of Figure 1 are examples of traditional histology where a dye, stain or marker is introduced either into the cochlea directly or systemically by a perfusion through the vascular system. Traditional biological markers are used to illustrate the structure of the tissue and do not reflect on the actual functional status of the tissue. Figure 4 is a picture of cochlear tissue stained with dichlorofluorescein (DCF), a marker that reacts with free radical molecules and produces a fluorescent green precipitate. The pattern of DCF labeling provides a qualitative assessment of the prevalence and pattern of free radical activity.

Figures 7 and 8 show the nuclei of OHC after exposure to noise and are stained with propidium iodide (PI), a chemical that penetrates the membrane of the nucleus of unfixed tissue when the nucleus is damaged and the cell is about to begin the death process. Note, in Figs 7 and 8, the tissue was fixed, therefore, PI enters all nuclei. Consequently, the histological assessment is limited to documenting the shape of the nucleus and its potential viability.

Figures 13 and 17 use double staining with both DCF and PI to show the free radical activity in the samples overlaid across the stained OHC that are still alive and the unstained spaces where the dead OHC had been.

Tutorial box 2.

induced changes in blood flow (using a variety of noise exposures and blood flow detection techniques) have found that noise decreases CBF (Lamm & Arnold, 2000; Miller, Brown, & Schacht, 2003; Perlman & Kimura, 1962; Thorne & Nuttall, 1987), but it should be noted that the exact role of CBF in noise-induced ROS formation is still unclear. A recent study found that a product of lipid peroxidation (8-isoprostaglandin $F_{2\alpha}$) can lower CBF and that a blocker for 8-isoprostaglandin $F_{2\alpha}$ can inhibit noise-induced reductions in CBF. These findings suggest that noise-induced ROS and ROS activity might be causing the reductions in CBF after noise rather than ROS resulting from the CBF changes. Regardless, it is possible that the noise-induced CBF changes are exacerbating ROS formation in the cochlea, if not causing them initially.

NECROSIS AND APOPTOSIS

If ROS can cause cochlear damage and loss of sensory cells, through what mechanisms do they do so? ROS and free radicals are capable of damaging DNA, breaking down lipid and protein molecules, and triggering cell death (Halliwell & Gutteridge, 1999), all of which can contribute to the HC lesion and loss of function seen after noise. An understanding of the biological steps in cell death may lead to the development of methods for prevention. The following sections will discuss the contribution of both necrotic and apoptotic cell death in the cochlea after noise exposure.

NECROSIS

Cell death occurs through one of two processes, either as necrosis or apoptosis (Kerr, Wyllie, & Currie, 1972). Necrotic cell death is a passive form of cell death and is often observed after gross physical or chemical insult. Necrosis is associated with cell swelling, which eventually results in the rupture of the cell and the spillage of the cell's contents, causing damage to surrounding tissue and initiation of an inflammatory response. Necrosis and the attendant inflammatory response typically results in the death of groups of cells. Before the work published by Kerr et al. (1972), it was thought that all cells died by necrosis. Necrosis can occur in the cochlea after traumatic noise exposure. The swollen cells that are found in the cochlea after traumatic impulse noise are examples of necrotic cell death. (See Fig. 6A for a schematic of necrotic cell death.)

Inside the Mitochondria

Mitochondria are the organelles in cells that are responsible for providing energy for the cell through the respiration of glucose using oxygen. The process occurs in three phases. In each phase, molecules of adenosine diphosphate (ADP) have a third phosphate group added to them to create adenosine triphosphate (ATP). Each addition of a phosphate group is called phosphorylation. The process stores a great deal of energy in each molecule of ATP, making ATP the true "battery' of the cell. Conversion of ATP to ADP, by removing one of the phosphate groups, releases a great deal of stored energy that then is used to power the cell.

Aerobic respiration occurs in three phases:

1) Glycolysis: The initial process in which the glucose molecule is broken down into two molecules of pyruvate.
2) The Krebs' cycle: A series of reactions in which electrons from pyruvate are used to generate ATP from ADP and to generate the high-energy molecule NADH from NAD^+.
3) The electron transport chain: The final stage of respiration, this is a series of reactions in which the energy state of the electrons in NADH is systematically lowered through a series of reactions with intermediary molecules. Many molecules of ATP are generated. Superoxide is created from oxygen as an intermediate "carrier" for electrons. A great deal of toxic superoxide is thought to come from leaking from the electron transport chain when the process occurs inefficiently due to heavy metabolic demand on the mitochondria and/or lack of oxygen supply.

The information in this box was adapted from Karp (1996).

Tutorial box 3.

APOPTOSIS

Apoptotic cell death plays a vital role in normal development. Apoptosis may occur as a means for the body to neatly eliminate unwanted or damaged/dying cells that could potentially damage neighboring healthy cells. These unwanted cells include excessive cells during development and old and damaged cells in adult tissue (Majno & Joris, 1995). However, over the past few years, our understanding of apoptotic cell death has lead to the discovery of a "dark side" of apoptosis, in which apoptosis is initiated at the wrong time and crucial cells die off (reviewed by Green & Reed, 1998). This form of apoptosis has been found to occur in a number of pathologies, including stroke, heart failure (Cheng, Deshmukh, D'Costa, et al., 1998; Hara, Friedlander, Gagliardini, et al., 1997; Namura, Zhu, Fink, et al., 1998), Alzheimer's disease (Cotman & Anderson, 1995; Kim, Pettingell, Jung, et al., 1997; Roperch, Alvaro, Prieur, et al., 1998), and autoimmune disease (Thompson, 1995). Apoptosis is an active, highly regulated process that consumes energy. Through the activation of a family of specific enzymes called caspases, the cell systematically disassembles. Throughout the process of apoptosis, the cell membrane remains intact, and the cell condenses and pulls away from neighboring cells result-

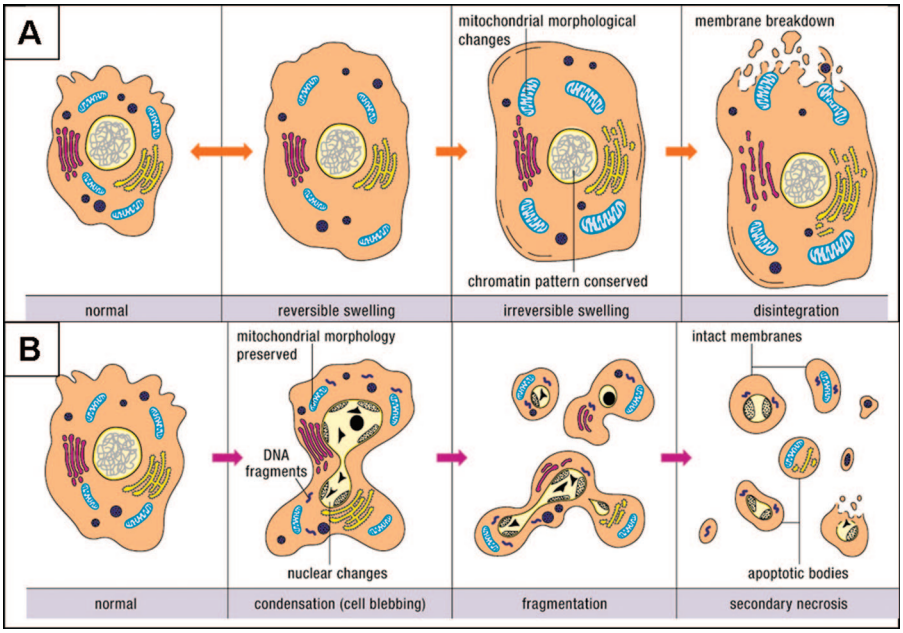


Fig. 6. ***A,*** **Schematic of necrotic cell death.** ***B,*** **Schematic of apoptotic cell death. From** ***Apoptosis and Cell Differentiation, 3rd Edition,*** **published online by Roche Applied Science.**

ing in minimal damage to surrounding tissue. (See Fig. 6B for a schematic of apoptotic cell death.)

The designation of a cell as apoptotic or necrotic is dependent on the observation of morphological and/or biochemical markers. Morphologically, apoptotic cells exhibit shrunken nuclei and subsequently break into apoptotic bodies or broken off fragments of the cell.

Apoptosis and Necrosis in the Noise-Exposed Cochlea

As mentioned earlier in the text, OHC loss is a prominent pathological change in the noise-exposed cochlea. The temporal pattern of OHC loss is well documented in the cochlea; OHC lesions develop during a noise exposure and continue to expand for days after the noise exposure (Bohne, 1976; Fredelius, 1988; Hamernik et al., 1986; Hu, Henderson, & Nicotera, 2002). Since OHC die over a relatively long time period, knowledge of the mechanisms of cell death may lead not only to prevention but also rescue after a noise exposure.

The literature has described morphological changes in HC in response to a traumatic noise exposure (Meyer & Biedermann, 1980; Spoendlin, 1971; Ward, 1980), including morphologic nuclear changes that are correlated with irreversible cell damage. As stated earlier, cell swelling is characteristic of a cell undergoing necrotic cell death. The presence of swollen OHC immediately after a traumatic exposure led to the belief that necrosis was the primary mode of cell death in the noise-exposed cochlea (Saunders, Dear, & Schneider, 1985). It was not until recently (Hu, Guo, Wang, et al., 2000; Hu et al., 2002) that the existence of apoptosis in the noise-damaged cochlea was described. In a systematic study of cell death after noise exposure (105-dB octave band centered at 4 kHz), Hu et al. (2002) reported the coexistence of necrotic and apoptotic HC. Figure 7 shows isolated examples of normal, apoptotic (shrunken), and necrotic (swollen) cells found in the cochlea immediately after a 1-hr noise exposure. Figure 8 shows a larger perspective of the organ of Corti 1 hr after a damaging noise exposure, with missing, apoptotic, and necrotic cells (Hu et al., 2000; 2002).

Previous experiments (Bohne, 1976; Hamernik, Turrentine, Roberto, et al., 1984) have reported that HC die for as many as 30 days after the exposure.

Sources of ROS

As stated earlier, ROS comes from many sources in the body. With molecular oxygen (O_2) being in such great supply, there is an abundant supply of oxygen to be converted into various ROS.

1) Mitochondria: Mitochondria are the organelles that use oxygen to metabolize glucose to provide cells with their required energy. Superoxide is used as an intermediate molecule in the Krebs' cycle and the electron transport chain. These molecules are quickly neutralized in a normal functioning mitochondrion by conversion into O_2, CO_2, or H_20. In an over-driven mitochondrion, superoxide molecules can escape and collect in the cell.

2) Enzymatic reactions: Some enzymes, including xanthine oxidase and NADPH oxidase, will catalyze reactions of hydronium ions (H^+) with O_2 to create superoxide.

3) Ischemia/reperfusion: A state of decreased blood flow to a certain tissue or organ is known as ischemia. Following a period of ischemia, blood flow returns to the deprived population of cells. This is called reperfusion. During ischemia, cells are deprived of oxygen, leading to greater mitochondrial taxation and increased leakage of superoxide. During reperfusion, there is an abundant supply of oxygen to be used in conversion to more superoxide, or to react with the existing superoxide to create other ROS.

4) Excitotoxicity: A condition in which exposure to large amounts of excitatory neurotransmitter (glutamate, in the cochlea) leads to cell death. The excitatory neurotransmitter causes heavy aerobic respiration in the mitochondria, leading to leakage of superoxide from the Krebs' cycle and electron transport chain.

Tutorial box 4.

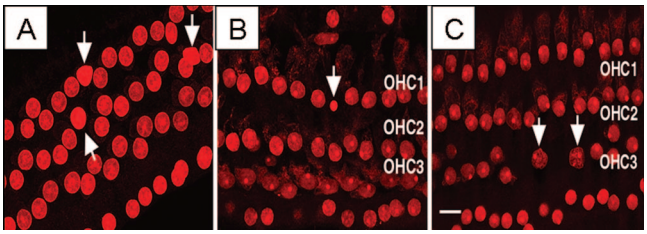


Fig. 7. A chinchilla organ of Corti stained with propidium iodide (see tutorial box 2) immediately after a 1-hr, 4-kHz narrow-band noise exposure of 110-dB SPL. Shown are normal OHC nuclei (*A*), an example of apoptotic nuclei (*arrow* in *B*), and an example of necrotic nuclei (*arrows* in *C*).

Thus, it would be of interest to know the mechanism responsible for the prolonged period of cell death. A useful technique for such a goal is the in vivo fixation procedure where the cochleae of a subject are fixed at different times after a noise exposure. This permits examination of how the damage to the cochlea progresses after the noise exposure, providing a perspective on how cochlear pathology progresses after noise exposure (Bohne, Harding, Nordmann, et al., 1999). Hu et al. (2002) exposed chinchillas to noise and fixed one ear at 6 hrs and the other at 24 hrs. The cochleae were labeled with propidium iodide (see tutorial box 2) to provide a

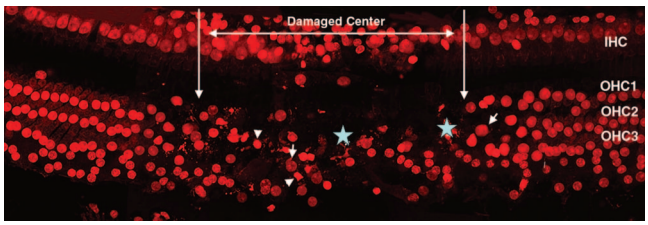


Fig. 8. Pattern of organ of Corti damage in a chinchilla 30 minutes after exposure to impulse noise of 75 pairs of simulated M-16 gunfire at 155-dB pSPL. Noise lesion is localized to a relatively small area of the cochlea where OHC are either missing, apoptotic (*arrowhead* or *asterisk*), or necrotic (*arrow*).

Endogenous antioxidant pathways
To control the levels of ROS and free radicals in the body, the body has a number of antioxidant molecules. The list of antioxidant molecules includes antioxidant proteins (e.g. albumin), antioxidant enzymes (e.g. catalase and superoxide dismutase), vitamins (e.g. Vitamin C and Vitamin E), and small molecules antioxidants (e.g. glutathione).
A brief review of selected antioxidant pathways follows:
Superoxide dismutase (SOD): SOD catalyzes the conversion of Superoxide into Hydrogen Peroxide (H_2O_2).
Catalase: Catalase catalyzes a reaction that converts two molecules of Hydrogen Peroxide to two molecules of water and a molecule of O_2. Thus, the potentially dangerous H_2O_2 is converted into harmless molecules.
Glutathione: The complex glutathione pathway is a key regulator of ROS in the body. It is extremely active in regulating the ROS intermediates formed in the mitochondria (Tutorial Box 2). It exists in a reduced form (GSH) and an oxidized disulfide form (GSSG). The GSH molecules can react with the hydroxyl radical ($OH^{\cdot}$) to form $GS^{\bullet}$ and water. The two $GS^{\bullet}$ molecules then join together to form GSSG. 2 GSH molecules can also convert H_2O_2 into two water molecules in a reaction catalyzed by glutathione peroxidase. GSSG molecules can then be recycled back to GSH molecules with glutathione reductase.

Tutorial box 5.

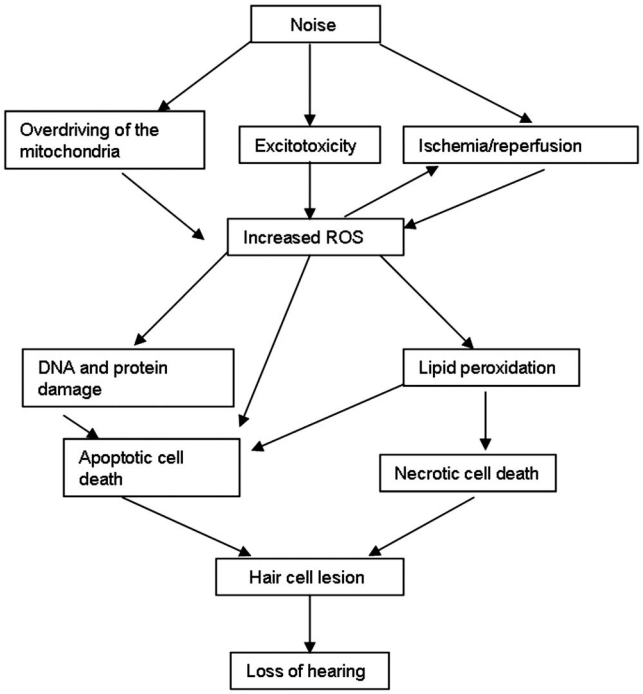


Fig. 9. Potential pathological pathways associated with damage from a noise exposure.

perspective on the nuclear size and stained for caspase-3, an enzyme associated with the final step of apoptosis. Their results confirmed the existence of apoptosis in the noise-exposed cochlea and indicated that both apoptosis and necrosis are initially involved in the creation of the OHC lesion, but with the passage of time, apoptosis is responsible for the expansion of the cochlear lesion towards the base of the cochlea. The fact that OHC death continues for days after the exposure and that cells die through both apoptosis and necrosis raises several questions, one of which is, What are the possibilities for interventions to prevent or rescue HC from death after noise exposure?

Summary of Noise-Induced Cell Death

Clearly, the pathways that bridge traumatic noise exposure and the resulting cochlear lesions are numerous and complex. Figure 9 shows a schematic of a variety of pathways through which noise can cause increased ROS and subsequent cochlear damage and loss of hearing. In the model, noise stresses the cochlea metabolically and mechanically at several points, leading to several different forms of damage. At the level of the HC, noise can lead to overdriving of the mitochondria, excitotoxicity at the junctions between the IHC and afferent auditory nerve fibers, and ischemia/reperfusion effects on the cochlea's blood supply. Each of these actions can lead to increases in ROS, which can damage DNA and the

cell membrane and act as a putative trigger for apoptosis. The end result is cell death from a combination of necrosis and apoptosis. Such a model is certainly not complete and not nearly as detailed as a comprehensive model would be. There is no doubt that the model will be expanded on as the knowledge base of NIHL, ROS, and cell death signaling pathways continues to grow.

INTERVENTIONS

As the pathways through which noise causes cochlear damage and loss of function become clearer, so do the possibilities for different approaches to intervene and reduce the damaging effects of traumatic noise. Using Figure 9 as our model of noise-induced cochlear damage, four points of intervention will be discussed: (1) restoring the normal balance of ROS with antioxidants or substrates for antioxidant synthesis; (2) limiting the amount of lipid peroxidation that occurs in the organ of Corti; (3) maintaining adequate CBF during and after noise; and (4) inhibiting pathways to apoptotic cell death to preserve HC.

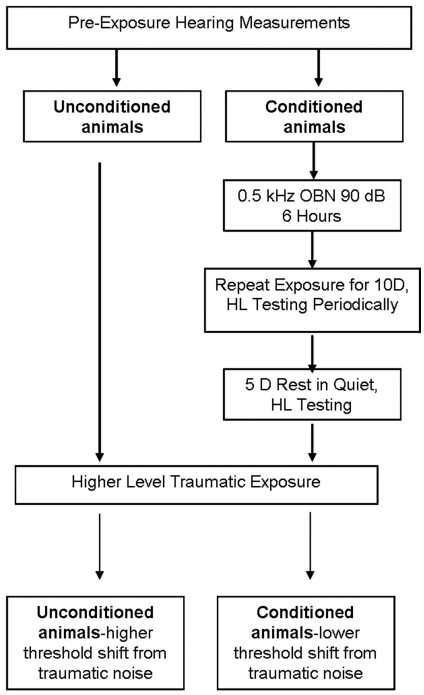


Fig. 10. A schematic of noise conditioning or acquired resistance to noise.

(1) ROS/ANTIOXIDANT BALANCE

Antioxidants are molecules that scavenge ROS and convert them to less dangerous molecules. A series of experiments, from a number of laboratories, show that increasing cochlear antioxidant supplies can substantially prevent HC damage and hearing loss. Antioxidant levels can be increased in two ways. The first is endogenously using sound conditioning. The second is through application of exogenous antioxidant molecules either directly into the cochlea or systemically into the body.

Sound conditioning refers to the acquired resistance to NIHL caused by beforeexposure to lower-level nontraumatic noise (Campo, Subramaniam, & Henderson, 1991; Canlon, Borg, & Flock, 1988; Henselman, Henderson, Subramaniam, et al., 1994). An example of the conditioning paradigm is shown in Figure 10. In this conditioning paradigm, chinchillas were exposed to a 500-Hz octave band noise at 95-dB SPL for 10 days. Hearing was measured before and after each day of the conditioning exposure. After the 10 days of conditioning exposures, the subjects were given 5 days of quiet, and their hearing sensitivity recovered. Finally, the chinchillas were exposed to the same noise at 110 dB SPL for 2 days. At the same time, a control group of animals with no history of noise exposure was exposed to the same 110-dB SPL noise. The conditioned animals had progressively less development of TTS during the 10 days of nontraumatic noise exposure. The conditioned subjects completely recovered during the 5-day quiet rest period. Finally, they were exposed to a high-level traumatic noise exposure and developed 10 to 20 dB less PTS than the nonconditioned subjects after the high-level traumatic exposure. Conditioning stimuli other than sound, including restraint conditioning (Wang & Liberman, 2002) and heat conditioning (Yoshida, Kristiansen, & Liberman, 1999), have also proven effective in reducing susceptibility to noise.

The protection developed by the prophylactic, or conditioning, noise exposure is a reliable phenomenon. In other experiments, the time between the 10-day conditioning exposure and the traumatic exposure was increased to 60 days. Even with this large time gap between exposures, there was still a large measure of protection from the conditioning (McFadden, Henderson, & Shen, 1997). The mechanism of the conditioning effect may be related to increased levels of antioxidant enzymes in the cochlea caused by the prophylactic exposure. Jacono, Hu, Kopke, et al. (1998) repeated the conditioning paradigm described in Figure 10 but also sampled tissue from stria vascularis and the organ of Corti from the subjects and analyzed them for three antioxidant enzymes: glutathione reductase, γ-glutamyl cysteine synthetase, and catalase. (See tutorial box 5 for a description of these enzymes and their significance.) Figure 11 shows the Jacono et al. (1998) experimental plan and shows how each of the enzyme levels is elevated compared with a nonex-

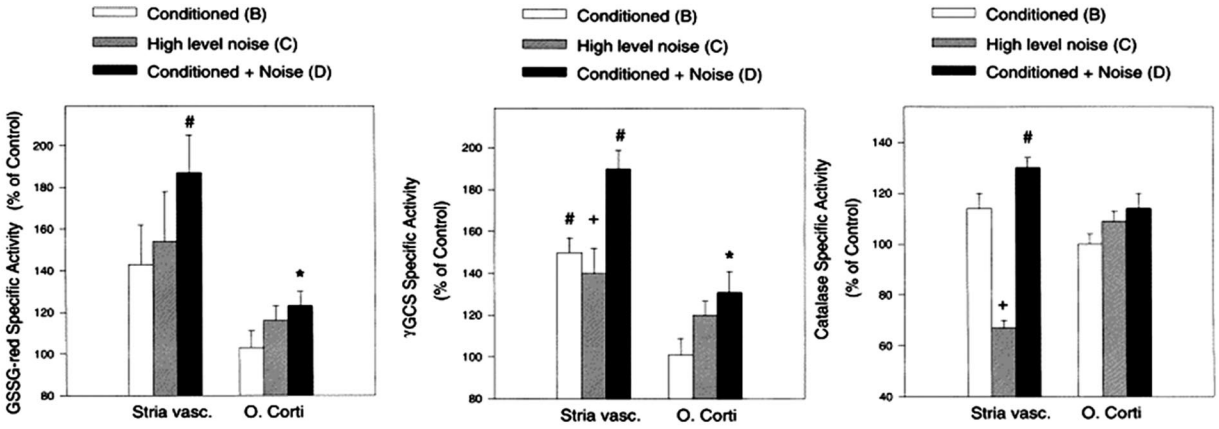


Fig. 11. Experimental groups and results from Jacono et al. (1998), using the chinchilla animal model. Levels of cochlear glutathione reductase (left panel), γ-glutamyl cysteine synthetase (middle panel), and catalase (right panel) after conditioning noise (500-Hz octave band at 90-dB SPL for 6 hrs/day for 10 days), traumatic noise (500-Hz octave band at 105-dB SPL for 4 hrs), and both conditioning and traumatic noise. All of the antioxidants show their greatest activity after the combination of conditioning and traumatic noise, suggesting that protection from traumatic noise as a result of conditioning may have antioxidant upregulation as an underlying mechanism. *,#,+Significant differences relative to unexposed controls. From Jacono et al. (1998).

posed control group. One group only received the 10-day conditioning exposure (without the traumatic exposure). The second group received the high-level traumatic exposure only, and the third group received both the conditioning and traumatic exposures. Interestingly, all three noise exposure paradigms resulted in increased levels of the enzymes in the stria vascularis and the organ of Corti. However, the biggest increase in the enzymes was found in the group that had both the conditioning and high level traumatic exposure (solid bars in Fig. 11). Since conditioned subjects from previous experiments have increased resistance to noise, then it is reasonable to assume that the conditioning protection is due to increased endogenous levels of antioxidants and/or antioxidant enzymes.

Given that increased endogenous antioxidants and antioxidant enzymes lead to greater resistance to NIHL, the next logical step is to explore whether treatment with exogenous antioxidants also leads to increased resistance to noise. Hu, Zheng, McFadden, et al. (1997) applied a 10-μL drop of R-phenylisopropyladenosine (R-PIA) to the round windows of chinchillas, with a drop of saline applied to the other round window to serve as an internal control. R-PIA induces increases in the antioxidant glutathione and antioxidant enzyme superoxide dismutase (SOD) (Maggirwar, Danraj, Somani, et al., 1994) (see tutorial box 5), as well as promoting blood flow and inhibiting glutamate excitotoxicity (both diminished blood flow and glutamate excitotoxicity are possible causes of ROS formation). The pervasive physiological effects of R-PIA make it impractical to use the drug systemically (which subsequently renders it impractical for much clinical use), but as seen in Figure 12, ears treated with R-PIA locally on the round window membrane had essentially the same TTS immediately after the 105-dB SPL 4-kHz octave band noise exposure but showed less PTS in the 4- to 16-kHz frequency region (Hu et al., 1997). In addition, the R-PIA–treated ears had significantly fewer missing OHC. R-PIA has also been shown to protect against impulse noise trauma (Hight, McFadden, Henderson, et al., 2003).

The protective value of antioxidants is difficult to assess with R-PIA, given that R-PIA can influence the potential noise trauma by stabilizing CBF and reducing excitotoxicity in the IHC, as well as increasing antioxidants. The protective value of antioxidants is more clearly shown by Hight et al. (2003), who used glutathione monoethyl ester (GSS). GSS is a precursor molecule to glutathione that can be readily absorbed into cells. Application of GSS onto the round window provided 10 to 20 dB of PTS protection from impulse noise in the frequency range from 0.5 to 16 kHz (Hight et al., 2003).

Studies that used antioxidants that were applied through the round window (Hight et al., 2003; Hu et al., 1997) showed that the technique of directly increasing cochlear antioxidants effectively reduced NIHL. However, round window administration is not a very practical technique clinically, certainly not for repeated noise exposures. Seidman, Shivapuja, & Quirk (1993) systemically injected rats with either allopurinol, an inhibitor of ROS production, or superoxide dismutase–poly ethylene glycol, an ROS scavenger. Both of the drug-treated groups of animals showed less compound action potential

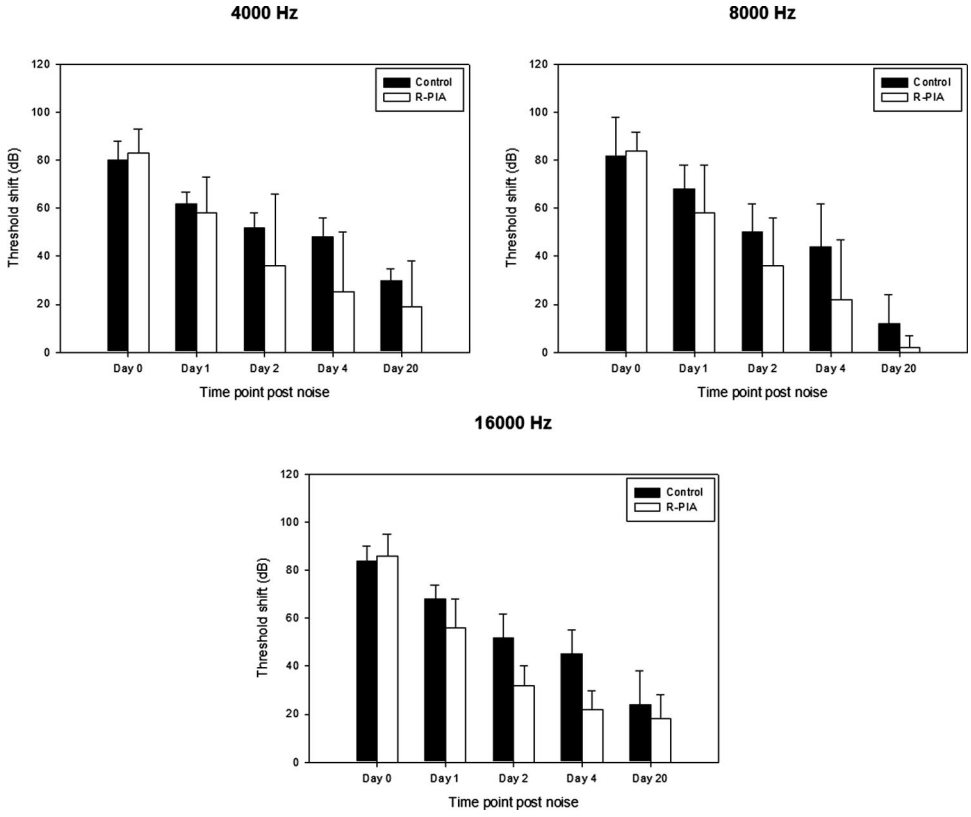


Fig. 12. Threshold shift in chinchillas from a 105-dB SPL, 4-kHz octave band noise of 4 hrs' duration. White bars represent R-PIA treated ears. Dark bars are control ears. Error bars are standard deviations. Adapted from Hu et al. (1997).

and cochlear microphonic threshold shift in response to a 90-dB SPL broad-band noise exposure of 60 hrs' duration (Seidman et al., 1993). Yamasoba, Schacht, Shoji, et al. (1999) gave systemic injections of mannitol, a scavenger of the hydroxyl ($OH^{\bullet}$) radical, to guinea pigs before exposing them to a 4-kHz octave band noise at 115-dB SPL for 4 hrs. The mannitol-injected groups showed lower threshold shift after the noise than did saline controls (Yamasoba et al., 1999). Ohinata, Yamasoba, Schacht, et al. (2000b) gave systemic injections of glutathione monoethyl ester to guinea pigs at several time points before and after a 5-hr, 115-dB SPL 4-kHz octave band noise. The guinea pigs had been living on a low protein diet that limited their ability to produce endogenous glutathione. The animals on the low protein diet had significantly higher threshold shifts 10 days after the noise exposure than the animals that had a normal protein diet and the low protein diet animals that had been given injections of glutathione monoethyl ester. The investigators concluded that glutathione was a key factor in the cochlea's protection against noise (Ohinata et al., 2000b). Kopke, Weisskopf, Boone, et al. (2000) administered intraperitoneal injections of *n*-L-acetylcysteine (LNAC) and salicylate to chinchillas that underwent noise exposure. LNAC has antioxidant properties and increases levels of glutathione. Salicylate can scavenge the hydroxyl radical. Given before the noise exposure, the LNAC/salicylate combination provided 20 to 35 dB of PTS protection and an OHC lesion that was 50% smaller compared with saline controls in the frequency range of 1 to 8 kHz after a 4-kHz octave band noise at 105-dB SPL for 6 hrs. When the injections were given at time intervals after the noise exposure, the protection effect was still present but less pronounced, suggesting a narrow time interval after noise exposure during which ROS do a significant amount of damage (Kopke, Weisskopf, Boone, et al., 2000). Finally, Kopke, Coleman, Liu, et al. (2002) gave a series of intraperitoneal injections to animals with one of two different drugs: acetyl-L carnitine (ALCAR) or D-methionine (DMET). ALCAR was given to improve mitochondrial respiration efficiency, thus leading to decreased ROS production during noise. DMET was given to increase the levels of available cochlear glutathione. Both drugs provided nearly 100% protection against noise-induced PTS, OHC loss, and IHC loss (Kopke Coleman, Liu, et al., 2002).

Of particular clinical interest are the findings of Kopke et al. (2000) that showed that antioxidant drugs can be administered after the noise exposure occurs, and protection can still be seen. Thus, a rescue effect can occur using these drugs because ROS are still damaging cells for a period of time after the noise exposure has ended. Further study of the rescue phenomenon is needed, including infor-

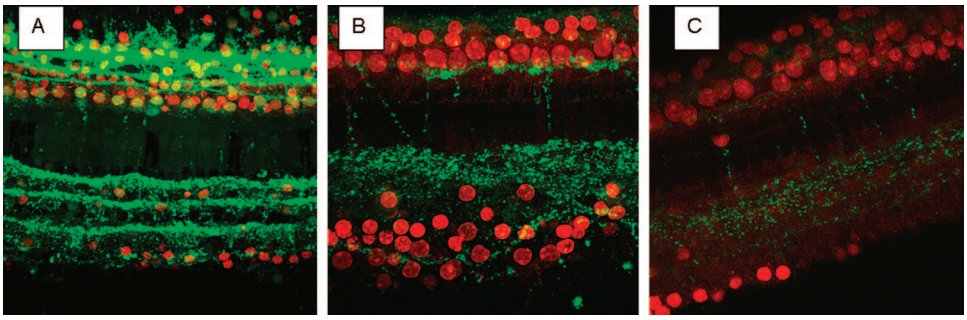


Fig. 13. Organ of Corti at 30 minutes (A), 2 days (B), and 4 days (C) after noise. Samples are stained red with propidium iodide (PI) to show OHC viability and fluorescent green with DCF to label for free radical activity. Note that as time increases, free radical activity decreases but is still discernible 4 days after the exposure (C).

mation on how long a period of time after the noise exposure ends antioxidants can be given to rescue the cochlea from damage. Van Campen, Murphy, Franks, et al. (2002) recently found evidence of oxidative DNA damage 8 hrs after an intense noise exposure, leading to the hypothesis that the 8 hrs after a noise exposure may be an important period for antioxidant intervention (Van Campen et al., 2002). Additionally, continued ROS formation has been found for 2 wks after noise exposure, with a maximum at 7 to 10 days, suggesting that antioxidant intervention may be beneficial for a substantial period of time after the noise exposure (Yamashita, Jiang, Schacht, et al., 2004). The critical window(s) for antioxidant intervention may depend on the length and intensity of the noise exposure.

The results with systemic drug administration are the most encouraging for clinical application. Theoretically, intraperitoneal or intramuscular injections can be used to administer drugs to humans in the clinical setting, but, realistically, the protection scheme is limited by the invasiveness of the injections. The development of an orally effective antioxidant intervention plan would increase the likelihood of clinical application.

(2) Lipid Peroxidation and Sustained Hair Cell Loss

As stated earlier, lipid peroxidation is a process through which ROS and free radicals break down lipid molecules. Lipids are a major component of the cell membrane. Thus, ROS and free radicals can break down cell membranes through lipid peroxidation, leading to cell death. Ohinata et al. (2000a) showed increases in lipid peroxidation in the cochlea after exposure to noise. Therefore, pharmacological interventions have been directed at interrupting the lipid peroxidation process to preserve HC. Quirk, Shivapuja, Schwimmer, et al. (1994) injected rats before and then during a 60-hr noise exposure with a lazaroid, a drug that inhibits lipid peroxidation. The injected rats showed lower TTS than noise-exposed animals that received only the drug's vehicle. The results implicate lipid peroxidation as a mechanism of NIHL (Quirk et al., 1994). More recently, a series of drugs that reduced lipid peroxidation effects in the organ of Corti were also found to limit noise-induced threshold shift. Those drugs that lowered lipid peroxidation in cochlear tissues outside the organ of Corti were not as effective in attenuating NIHL (Ohinata, Miller, & Schacht, 2003).

As stated earlier, lipid peroxidation is a self-perpetuating process that may be contributing to the expansion of the HC death lesion after noise. Figure 13 shows the evidence of lipid peroxidation in the cochlea after a noise exposure. The cochleae were stained with DCF (green fluorescence) and PI (red fluorescence) immediately after (panel A), 2 days after (panel B), and 4 days after (panel C) noise exposure. In panel A, heavy free radical activity (lipid peroxidation) is visible in the region of the OHC (the green fluorescence in the lower half of the ure with scattered red OHC nuclei). Note that by 4 days after noise, most of the OHC are missing, but there is still evidence of free radical activity in the neural network crossing the tunnel and at the OHC region (lower half of the ure). If lipid peroxidation continues after the noise exposure, as shown in Figure 13, pharmacological inhibition of lipid peroxidation may be a possible method for rescue of hearing after noise exposure, much in the way antioxidants were used by Kopke et al. (2000; 2002) after the noise exposure to preserve hearing. Pharmacological intervention aimed at directly inhibiting lipid peroxidation has yielded excellent results for protection against noise (Ohinata et al., 2003; Quirk et al., 1994).

(3) Cochlear Blood Flow

A third point of intervention against NIHL (Fig. 9) may be prevention or at least moderation of the cochlear ischemia/reperfusion associated with noise exposure. As discussed earlier, noise may be inducing alterations in CBF that can contribute to the increased levels of ROS in the cochlea (Miller et al.,

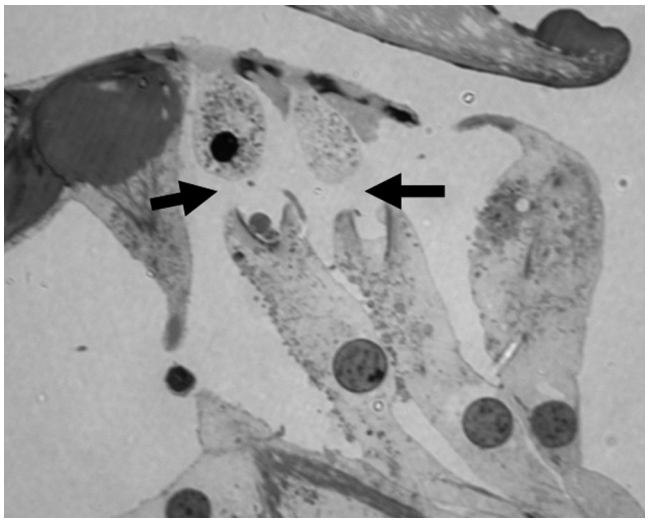


Fig. 14. Cross section of the OHC region of the organ of Corti of a chinchilla after impulse noise exposure (75 pairs of simulated M-16 gunfire at 155-dB pSPL). Notice the detachment of the first OHC from its supporting Deiters' cup.

2003; Yamane et al., 1995). Drugs that promote blood flow have been given as a possible protection against NIHL, with mixed results. Miller & Dengerink (1988) reviewed experiments on noise and CBF. They described how CBF can be increased experimentally in a number of ways to try to protect against NIHL. Cardiac output can be increased, leading to increased blood flow throughout the body. Cochlear blood vessels can be dilated, leading to wider vessels through which blood can pass. Blood can be thinned by expanding the plasma content. Blood cells can also be made more flexible, allowing them to move through constricted spaces.

Pentoxifylline is a drug that renders the blood cells more flexible. Intravenous administration of the drug led to reduction in noise-induced TTS (Latoni, Shivapuja, Seidman, et al., 1996). Blood vessel constriction was still observed in the cochleae that underwent noise exposure, but the blood flow to the cochlea was preserved due to the pentoxifylline. Similar findings were obtained with sarthran, a blocker of angiotensin II receptors (Goldwin, Khan, Shivapuja, et al., 1998). Angiotensin II is a peptide that induces blood vessel constriction and is found to be elevated in the plasma after noise exposure. Blockade of angiotensin II receptors in the cochlear vascular system with sarthran led to maintenance of normal blood vessel diameter during noise, when control animals had substantial reduction in blood vessel diameter. Also, TTS was reduced in ears exposed to sarthran, especially in the low frequencies (Goldwin et al., 1998).

One of the mechanisms through which noise may be altering CBF is through activation of the sympathetic nervous system and its projections to the cochlea. Cutting of the superior cervical ganglion leads to reduced susceptibility to NIHL (Borg, 1982; Hildesheimer, Henkin, Pye, et al., 2002; Hildesheimer, Sharon, Muchnik, et al., 1991; Horner, Giraudet, Lucciano, et al., 2001). Although unconfirmed, the reduced susceptibility to noise seen in these animals may involve stabilized CBF during noise. Blockade of the receptors for norepinephrine, the neurotransmitter used by the cochlear sympathetic fibers, has been shown to increase CBF (Ohlsén, Baldwin, Nuttall, et al., 1991) and reduce noise-induced TTS (Hildesheimer, Muchnik, & Rubinstein, 1990), although it is unknown if the two effects are related.

Overall, it is still unclear how well noise-induced CBF changes can be manipulated with pharmacological intervention, nor is it clear what effect such manipulation might have on noise-induced ROS formation and NIHL.

(4) Apoptotic Cell Death

The fourth and final point of intervention to be discussed in this review comes at the level of the cellular signals responsible for apoptotic cell death. As mentioned previously, either intrinsic signals such as the overproduction of ROS or extrinsic signals such as the stressing of the cell junctions or toxic soluble factors surrounding the cell, trigger a series of intracellular signaling cascades that result in cell death. To date, little is known about the precise intracellular apoptotic pathways activated in the noise-exposed cochlea. Researchers believe that apoptotic cell death pathway in HC is most likely a multifaceted response. By examining the intracellular signaling pathways initiated in other cell types, researchers have begun to identify possible key players in cochlear apoptosis. One way of examining the function of various signaling components is to introduce specific cell signaling inhibitors and examine the effect on NIHL.

Pirvola, Xing-Qun, Virkkala, et al. (2000) examined the effects of CEP-1347, a selective c-*Jun*-N-terminal (JNK) inhibitor on noise-exposed guinea pigs. JNK, an intracellular signaling component in the apoptotic cell death pathway, has been shown to be activated in response to a variety of stressful stimuli, including ROS, in multiple cell types (Dérijard, Hibi, Wu, et al., 1994; Kyriakis, Banerjee, Nikolakaki, et al., 1994). CEP-1347 was delivered subcutaneously, starting 2 hrs before a 120-dB, 4-kHz, 6-hr noise exposure and was continued daily for 2 wks after the noise exposure. CEP-1347–treated animals showed significantly less PTS than controls. Similar effectiveness was found with D-

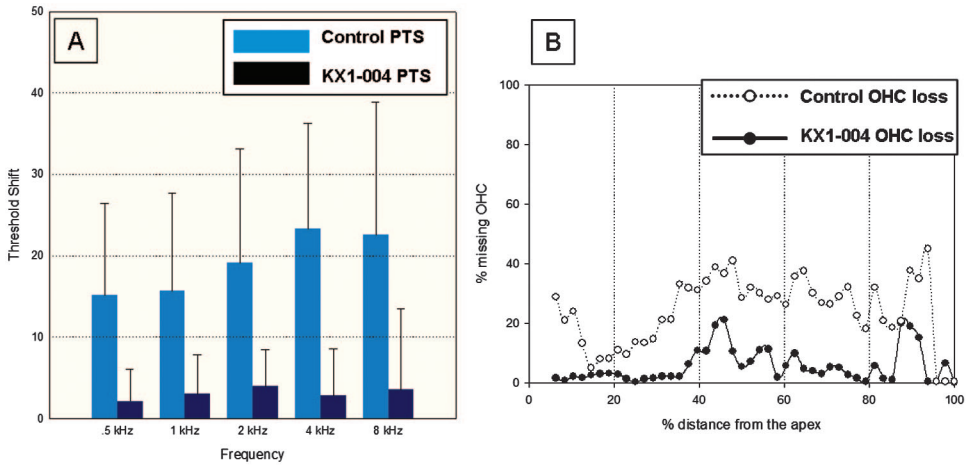


Fig. 15. ***A,*** **Threshold shift in chinchillas from an impulse noise exposure that consisted of 75 pairs of impulses of 155-dB pSPL intensity. Dark blue bars represent KX1-004–treated ears. Light blue bars are control ears. Error bars are standard deviations.** ***B,*** **OHC cochleograms from the same experiment. Dark circles represent KX1-004–treated ears. Open circles are control ears.**

JNK-1, which also disrupts the JNK pathway and inhibits apoptosis. Infusion of D-JNK-1 into the cochleae of guinea pigs nearly completely eliminated threshold shift from a 6-kHz pure tone of 120-dB SPL for 30 minutes (Wang, Van De Water, Bonney, et al., 2003). Riluzole, a neuroprotective agent that restricts excitotoxicity and apoptotic and necrotic cell death, was also effective in limiting PTS and HC loss in guinea pigs that received the same 6-kHz pure-tone exposure (Wang, Dib, Lenoir, et al., 2002).

Another intracellular signaling component examined recently is Src protein tyrosine kinase (Src) (Harris, Hu, Hangauer, et al., 2005). The examination of Src was undertaken due to its possible role in signaling both mechanical stresses (impulse related injuries) as well as metabolic changes (increases in ROS). Traumatic noise can stress or actually separate tight cell junctions (Ahmad et al., 2003; Hamernik et al., 1984). The binding of the cells to each other and to the extracellular matrix in which they reside regulates cell survival. Disruptions between cells and between cells and the extracellular matrix often lead to apoptosis (Frisch & Screaton, 2001). For example, noise can lead to the separation of OHC from their Deiters' cell cups. Figure 14 shows this detachment after an impulse noise exposure. Note that the OHC nucleus has already begun to shrink, indicating that the cell has entered the apoptotic cycle. If Src activation was involved in initiating apoptosis in these cells, then inhibition of Src should result in less PTS and HC loss. A study by Harris et al. (2005) used KX1-004, a potent inhibitor of Src activity, applied directly to the round window of the chinchilla before an impulse noise exposure. Ears pretreated with KX1-004 exhibited significantly less PTS (Fig. 15A) and OHC loss (Fig. 15B) than did untreated control ears.

In addition to the possible involvement of Src in mechanically induced cell death, Src may also be involved in the generation or propagation of ROS in the cochlea during and after noise. The role of Src in ROS production is complex, and in many models occurs in a multistep mechanism in which ROS can activate Src directly. Src activation can then lead to an increase in ROS production, which in turn can lead to an increase in Src activity, thereby amplifying the ROS activity (De Keulenaer, Chappell, Ishizaka, et al., 1998; Griendling &Ushio–Fukai, 2000; Mollnau, Wendt, Szocs, et al., 2002; Seshiah, Weber, Rocic, et al., 2002). If Src activation does play a role in increasing ROS production and, consequently NIHL, then inhibition of Src should decrease the amount of ROS activity and PTS and OHC loss after a noise exposure.

The Src signaling system appears to be involved in metabolic noise-induced HC loss. Harris et al. (2005) pretreated one ear of each chinchilla with KX1-004 before a 4-hr continuous noise exposure (4-kHz octave band noise at 106 dB), whereas the opposite ear was treated with the vehicle alone. Ears pretreated with KX1-004 had substantially less hearing loss (Fig. 16A) and HC loss than control ears (Fig. 16B). The protective effects of the Src inhibitor was achieved with doses of 1/100 to 1/1000 the concentration of antioxidants (Hight et al., 2003; Hu et al., 1997), suggesting that the Src inhibitor acts early in the cell death process and prevents the formation of ROS. Furthermore, ears pretreated with KX1-004 exhibited a decrease in DCF staining, a marker for oxidative stress, after a noise exposure (Fig. 17) (Nicotera, Hu, & Henderson, 2003a). These results clearly support a role for Src activation in noise-induced apoptotic pathways. The success of both CEP-1347 and KX1-004 represents an alternative therapeutic approach. Although the currently available research has shown protective effects when the drug is applied before the noise exposure, there are many reasons to believe that these types of therapeutic agents will be successful when the treatment is initiated after trauma. Further studies need

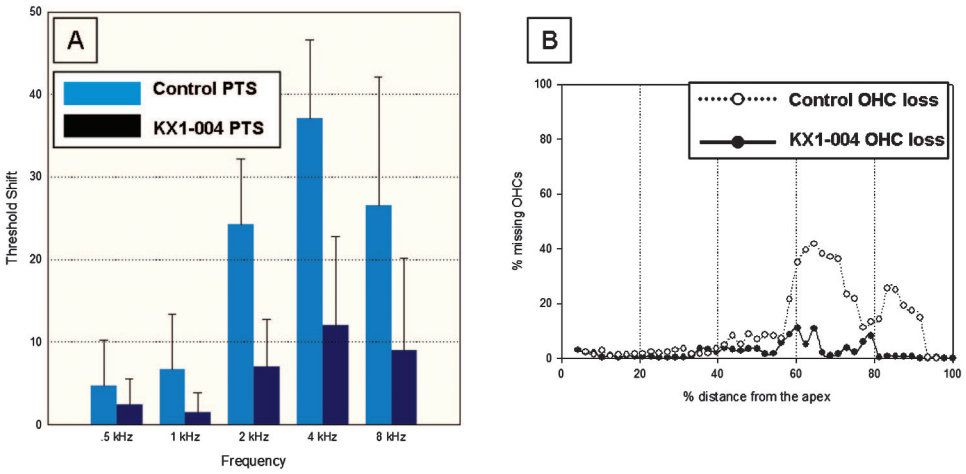


Fig. 16. *A,* Threshold shift in chinchillas from continuous noise exposure of 105-dB SPL, 4-kHz octave band noise of 4 hrs' duration. Dark blue bars represent KX1-004–treated ears. Light blue bars are control ears. Error bars are standard deviations. *B,* OHC cochleograms from the same experiment. Dark circles represent KX1-004–treated ears. Open circles are control ears.

to be conducted to determine the possibility of systemic application and to determine whether there are limiting side effects.

In addition to the Src and JNK signaling pathways, numerous other pathways are involved in the induction of apoptosis. These pathways may represent other avenues for intervention in noise-induced apoptotic cell death in the cochlea. The caspases enzyme cascade (mentioned briefly earlier) plays a key role in the execution of apoptotic cell death in the HC (Nicotera, Hu, & Henderson, 2003b; Van De Water, Lallemend, Eshraghi, et al., 2004). Inhibition of several of the key caspases may be an avenue for protection from noise-induced apoptosis. Though definitive results showing protection from noise using inhibitors of caspases have not been found, they have been proven effective in limiting apoptotic HC death from cisplatin (Van De Water et al., 2004; Wang, Ladrech, Pujol, et al., 2004) and they remain an intriguing potential pathway to protection from noise. The calpain enzyme pathway, a series of calcium-dependent enzymes that are involved in breaking down cells during apoptosis, has also been targeted for intervention to protect against noise (reviewed in Lefebvre, Malgrange, Lallemend, et al., 2002). Leupeptin, a calpain inhibitor, protected chinchilla HC (Wang, Ding, Shulman, et al., 1999) and reduced TTS (Salvi, Shulman, Stracher, et al., 1998) from a 48-hr traumatic noise exposure. Like the other apoptosis signaling pathways discussed, calpains represent an intriguing avenue for intervention to protect from noise-induced damage.

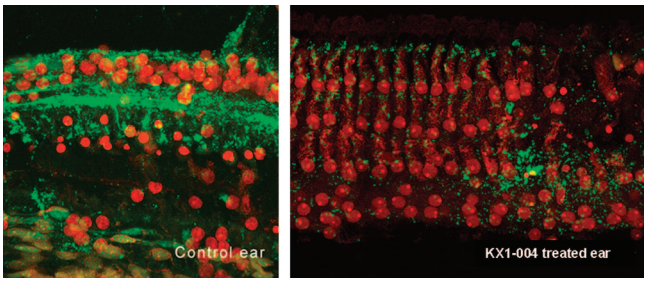


Fig. 17. Noise-exposed (75 pairs of simulated M-16 gunfire at 155-dB pSPL) chinchilla organ of Corti labeled with PI and DCF (see tutorial box 2). Left panel is a control ear that shows extensive free radical activity. Right panel is from an ear treated with KX1-004 on the round window. Note the greatly reduced free radical activity in the KX1-004–protected ear.

CONCLUSION

The last several years have brought significant advancements in our understanding of noise-induced cochlear damage, especially the importance of free radical expression in the cochlea. With those advancements have come a number of targets for intervention to preserve cochlear anatomy and function. The eventual goal remains finding practical means through which to preserve the hearing in humans who have been exposed to traumatic noise. Although there are reasons for optimism in this search, prevention of exposures through individual protective measures and education of the public and the audiological community regarding the hazards of noise remain the most effective means of limiting NIHL as an epidemic health hazard.

Oxidative Stress and Acquired Hearing Loss

Given the similarities in audiometric changes and cochlear pathology for hearing losses from noise, ototoxic drugs, and aging, it is not surprising that there may be a common factor underlying these seemingly different causes of hearing loss. There is growing evidence that the principles of ROS damage and multiple cell death pathways in the cochlea may be a common factor for hearing loss from aminoglycoside antibiotics (Sha & Schacht, 2000), ototoxic

anticancer drugs (Campbell, Rybak, Meech, et al., 1996), industrial solvents (Henderson, Yang, & Hu, 2004), and aging (Seidman, 2000). The story behind free radicals and the other forms of acquired hearing loss continues to be developed, and experiments using antioxidants to prevent cochlear damage and hearing loss from each of these insults may have exciting implications for future application.

As the body of research in the areas of acquired hearing loss continues to grow, it is hoped that applications for clinical practice will become more and more practical, and the benefit to the ever-increasing patient population will be tangible.

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